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(54) Title: NEW PRESERVATIVE COMPOSITION			
(57) Abstract			
<p>The invention is related to a composition intended for the preservation of green forage, fresh cuttings, grain or other organic material and for the control of micro-organisms, containing an ester of an unsubstituted or substituted benzoic acid. The composition comprises at least ester component (1) which is an ester of the unsubstituted or substituted benzoic acid with a C₁-C₉ alcohol, or mixture of such esters, and another ester component (2) which acts synergistically with said ester component (1) and which is an ester of an unaromatic C₁-C₂₀ carboxylic acid with a C₁-C₉ alcohol. The composition may also contain acid component (3) and other active ingredients.</p>			

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NEW PRESERVATIVE COMPOSITION

The invention relates to compositions suitable for preserving green forage, fresh
5 cuttings, grain or other organic material, and for controlling hazardous micro-organisms, containing an ester of unsubstituted or substituted benzoic acid.

Generally, when preserving green forage, the AIV process is used so that the pH of
the forage mass to be preserved is lowered to about 4 or less by adding acidic com-
10 pounds and by means of the lactic acid fermentation occurring in it. To this end, mineral acids, organic acids, specifically formic acid, acetic acid, and propionic acid, as well as acidic salts are used.

Patent publication FI63328 to Lampila discloses the use of benzoic acid as an addi-
15 tive in acidic preservatives for preserving green forage and the like, decreasing adverse fermentation and increasing the sugar content. The preservatives according to the publication contain 2 %-60 % of benzoic acid or a derivative, considered as benzoic acid.

20 Practice has proven that the content of benzoic acid in the preservative of the AIV process should be at least 5 wt-% for it to be sufficient in inhibiting the growth of hazardous microbes. However, it is difficult to use such high concentrations of benzoic acid in liquid preservatives. As it is slightly soluble, it causes difficult problems in crystallisation in storage vessels, resulting in blockage of measuring sprayer noz-
25 zles. Patent application FI920887 suggests stabilised compositions containing 5-30 wt-% of benzoic acid, comprising, e.g. formic acid, and aliphatic carboxylic acid containing at least 2 carbon atoms.

Patent publication PCT/FI93/00068 discloses stabile compositions containing 5-25
30 wt-% of benzoic acid or p-hydroxybenzoic acid or the salt or ester thereof which, in

addition to other possible components, contain formic acid, acetic acid, and/or propionic acid or their esters.

Patent application FI920886 discloses preservatives that contain benzoic acid or its salts and ethyl formate and, possibly, C₁ - C₃ carboxylic acids.

Patent publication GB 2 193 078 presents compositions for preserving moist hay, comprising acetic acid, propionic acid, or preferably sorbic acid which contains magnesium oxide, zinc oxide, and soluble Cu(II), preferably at a ratio of 100:10 - 20:1.

Publication J.Am.Soc.Brew.Chem. 37 (1979):3, pp. 140-144 explains, for preserving brewer yeast, the applicability of Na-benzoate, formaldehyde, and propionic acid as well as that of the following compounds: acetic acid/propionic acid, isobutyric acid/propionic acid, formaldehyde/propionic acid, formaldehyde/formic acid, and formaldehyde/acetic acid/propionic acid.

Patent publication DE 3 540 771 discloses a provision of formic acid esters of 1,2-propanediol in preserving animal forage and treating drinking water. The esters act on coliform bacteria in particular.

Patent publication JP 57 018 601 discloses the use of carboxyl acids and their derivatives (such as chlorides, esters, anhydrides, amides, and nitriles) and salts in solutions of 10⁻³ - 10⁻¹ M as pesticides against different plant diseases.

Patent publication US 3 745 027 discloses the preserving of foodstuffs, such as meat, fruits, and vegetables against moulding and bacterial germs, by cooking them in a solution containing 25-90 wt-% of polyalcohols and 0.5-1 wt-% of fungicide. The fungicides include sorbic acid, propionic acid, and benzoic acid, and their salts and esters.

Furthermore, patent publication US 3 404 987 discloses the use of propionic acid, sorbic acid, benzoic acid, essence niobe, ethyl benzoate, and the edible alkali salts and alkaline earth salts of the said acids to protect foodstuffs against micro-organisms.

The efficacy of the ethyl benzoate and the essence niobe in inhibiting the growth of *Aspergillus flavus* and *A. parasiticus* and in decreasing the amount of aflatoxin produced by these organisms is disclosed in publication *Applied and Environmental Microbiology*, Aug. 1980, pp. 352-357.

Publication *Food Microbiology*, 5 (1988) : 3, pp. 135-139, explains that a composition containing 1 % of acetic acid, 1 % of lactic acid, maleic acid, tartaric acid, or citric acid, 0.05 % of sorbic acid, 0.05 % of benzoic acid, and 3.5 % of salt, acts, in a salad whose pH is buffered to 4.8, as a bactericide with respect to *Salmonella* blockley, *Escherichia coli*, and *Staphylococcus aureus*, and is bacteriostatic with respect to *Streptococcus faecalis*.

Publication *International Journal of Food Microbiology* 22 (1994) : 2-3, pp. 127-140, explains that the combination of potassium sorbate and sodium benzoate slows down or inhibits the growth of *Salmonella* in milk or cheese when its pH is adjusted by means of propionic acid.

According to publication *Poultry International*, July 1994, pp. 40-42, mixtures of organic acids, particularly propionic acid and formic acid, or mixtures of organic acids and their ammonium salts, or salts of organic acids are used to prevent *Salmonella* in poultry forage. The products are either solutions or solids.

According to patent publication EP 275 958, solid forage for hens, cows, pigs, etc.,

containing 20 wt-% of moisture, at the most, can be preserved without drying by using a preservative that contains sorbic acid or sorbates together with a physiologically-harmless organic or inorganic acid or its salt.

- 5 According to US 5 234 703, the carcasses of dead animals, such as chickens, are disinfected by treating them with solutions containing, in addition to acetic acid, propionic acid, citric acid, and hydrochloric acid, also medium-chain fatty acids, such as C₁₀ fatty acids. The mixture is reported to have an effect on, e.g., *Salmonella typhirium* and *Escherichia coli*.

10

According to publication JAOCS 61 (1984) : 2, pp. 397-403, lauryl monoglyceride, i.e. monolaurine, is an efficacious antimicrobial which is suitable for foodstuffs. A mixture of monolaurine, Methylparaben, Propylparaben, and EDTA is well-known in preserving cosmetic protein-bearing emulsions.

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Preservative of green forage absorbed in natural zeolite or synthetic zeolite is known from patent SU 1 461 398. It contains 10-50 wt-% of organic acids, such as formic acid, acetic acid, and propionic acid, and 10-60 wt-% of zeolite while the rest comprise salts of inorganic acids or salts of water-soluble organic acids, such as Na-benzoate, Na-salicylate, or Na-acetyl salicylate.

20

PCT publication 91/11192 discloses a solid product containing acid and intended for the preservation of green forage or for an additive of animal forage, in which the acid is absorbed in siliceous earth, preferably in granular clay-bearing siliceous earth, 'moler earth'.

25

The above forage preservatives leave a lot to be desired, particularly when preserving predried green forage, whereby the grass is cut and left lying on the surface of the ground to be predried before embalming or siloing. During this stage, mould, bacteria, yeast and their germs may go into the forage from the ground so that the

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lot is easily spoiled.

Even though the compositions containing about 5-25 wt-% of benzoic acid component, disclosed in FI 920887 and PCT/FI93/00068, generally yield good results in preserving predried green forage, they still have defects. If sufficient amounts of expensive stabilising agents are not used, slight crystallisation may occur in the preservative during long-term cold-storage. It would also be advisable to decrease the amount of benzoic acid component, partly because of the expensive price; partly because it stains machines in such concentrations and forms a light-coloured, benzoic acid-bearing layer of dirt on the machine when drying, which is difficult to remove.

Another defect of present preservatives is frequently that, after the bale or the silo of forage has been opened, the forage that so far had been well-preserved, may deteriorate fast when its sugars start to ferment. It would be advisable to preserve the forage so that some sugars remain but, nevertheless, postfermentation is as minor as possible.

When not dried, the crop is preserved either as coarsely powdered or as whole wheat. In the former case, the moisture of the grain is about 30-35 % and the preservation is generally effected by using the same preservatives according to the AIV process as in the preservation of green forage. In whole grain preservation, the conventional preservatives for green forage are not effective enough but propionic acid, for example, is used, which is expensive. Therefore, it would be advisable to find more cost-effective preservative compositions also for grain preservation.

Furthermore, it would be good if the same preservative compositions could be used for other purposes as well, such as the controlling of Salmonella and other microbes in agriculture and forage production.

Moreover, it would be desirable to achieve the results of preservation according to the AIV process without needing to use as many or as strong acids, but accepting a higher initial pH instead.

- 5 It would also be desirable to decrease the corrosiveness of preservatives containing, e.g. formic acid, on the skin, clothes, and machines, and to make their pungent odour milder.

Surprisingly, it has been discovered that the defects listed above can be decreased to
10 a considerable degree when a preservative composition containing esters of unsubstituted or substituted benzoic acid is used as the preservative, comprising an ester component 1) which is the ester of an unsubstituted or substituted benzoic acid with $C_1 - C_9$ alcohol or a mixture of such esters, and an ester component 2) that acts synergistically with said ester component 1) and is the ester of unaromatic $C_1 - C_{20}$ carboxylic acid with $C_1 - C_9$ alcohol. Even though the references cited above present
15 the use of these components per se, it has not been realised that the components have a surprising, synergistic effect which was discovered through reliable comparative tests (cf. the examples of this application).

- 20 Thus the acid of the ester component 1) is an unsubstituted or substituted benzoic acid.

Benzoic acid and hydroxybenzoic acid are particularly advantageous. $C_1 - C_9$ alcohols of ester component 1) or 2) include primary and secondary saturated or unsaturated alcohols which possibly contain more than one hydroxyl group and whose
25 chain is either straight or branched. Saturated alcohols include, e.g., methanol, ethanol, n-propanol, isopropanol, butanols, particularly n-butanol and isobutanol, pentanols, hexanols, heptanols, octanols, particularly n-octanol and 2-ethylhexanol, and nonanols. Alcohols containing several hydroxyl groups include glycols, such as 1,2-propanediol, and triols, such as glycerol. Preferred alcohols comprise $C_1 - C_4$ alco-
30

hols, such as ethanol, propanol, and butanol. A viable aromatic alcohol is, e.g., benzoic alcohol.

The preferred esters of ester component 1) include methyl esters, ethyl esters, n-propyl esters, isopropyl esters, n-butyl esters, isobutyl esters, n-amyl esters, isoamyl
5 esters, n-octyl esters, or 2-ethylcapryl esters of benzoic acid or p-hydroxybenzoic acid. Even more preferred are methyl benzoates, ethyl benzoates, propyl benzoates, isopropyl benzoates, butyl benzoates, and isobutyl benzoates; particularly preferred are ethyl benzoate, propyl benzoate, and isopropyl benzoate.

10

The unaromatic $C_1 - C_{20}$ carboxylic acids of ester component 2) include saturated or unsaturated mono-, di-, or tricarboxylic acids which may contain hydroxyl groups. Such saturated monocarboxylic acids include formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, capronic acid, caprylic acid, 2-ethylhexanoic acid, capric acid, lauric acid, and myristinic acid. Di-
15 carboxylic acids include oxalic acid, malonic acid, succinic acid, glutaric acid, adipic acid, pimelic acid, and suberic acid with their alkyl and hydroxy derivatives. Hydroxycarboxylic acids include glycolic and lactic acids, saccharic acids, tartaric acid, maleic acid, and citric acid. Unsaturated acids include fumaric acid, maleic acid, sorbic acid, oleinic acid, linolic acid, linolenic acid, and ricinic acid.
20

Regarding the preservative effect, preferred esters of ester component 2) comprise esters which, when hydrolysing in their object of use, at the same time form acids which function as preservatives, the acids including formic acid, acetic acid, propionic acid, lactic acid, octanoic acid, 2-ethylhexylic acid, or sorbic acid; or alcohols
25 such as octyl alcohol, acting as a preservative.

Especially preferred esters of ester component 2) include propyl, isopropyl, butyl, and isobutyl esters of formic acid, propionic acid, and lactic acid; the most preferred
30 is propyl propionate.

The preservation power of ester component 2) alone may be unsatisfactory. Therefore, it is assumed that the synergy is based on the fact that this ester, independent of the application, improves the adhesion, the migration, and the absorption of ester component 1) on the surfaces of the material to be preserved and on those of the bacterium, yeast, or the like to be controlled. Such synergy had not been detected earlier.

The synergy of the preservative according to the invention is always materialised when there are present both the ester component 1), i.e., the ester of an unsubstituted or substituted benzoic acid with $C_1 - C_9$ alcohols, and ester component 2), i.e., the ester of unaromatic $C_1 - C_{20}$ carboxylic acid with $C_1 - C_9$ alcohol. According to one embodiment, the relative weight moieties of ester component 1) and ester component 2) in the composition range between about 10:1 - 1:5, preferably between about 4:1 - 1:2, most preferably between about 2:1 - 1:1.5.

It is obvious that the preservative composition according to the invention may also contain other preservative components. These include the preservatives listed in the preceding survey of the literary, therefore, the publications and their English equivalents mentioned in the survey are incorporated here by reference.

Furthermore, the application of the preservative composition of the invention intended particularly for the preservation according to the AIV process contains acid component 3). Its purpose is to lower the pH of the treated object to 4 or less. Regarding the invention, the quality of the acid is not critical. The acid component may be an organic or inorganic acid or a polyacid, or a composition that forms acid in a solution. According to one embodiment, the acid component contains organic $C_1 - C_{20}$ acid, and preferably $C_1 - C_4$ acid. Viable organic acids include particularly formic acid, acetic acid, propionic acid, and lactic acid, preferably formic acid, but other carboxylic acids or polycarboxylic acids, such as citric acid, can also be used.

Inorganic acids include hydrochloric acid, sulphuric acid, or phosphoric acid, preferably phosphoric acid. They may be partly neutralised. Compositions that form acids include inorganic or organic salts or acid anhydrides that react in an acid manner. The acid components can also be produced chemically in the course of the preservation according to the invention; for example, by using lactic acid fermentation controlled by enzymes and/or bacterium inoculation. The acid component is a formic acid, acetic acid, lactic acid and/or propionic acid, possibly neutralised, preferably formic acid, of about 10-100, preferably about 30-100, and more preferably 60-100 wt-%. According to one preferred embodiment, the preservative composition of the invention contains about 70-90 wt-% of formic acid, about 0-10 wt-% of orthophosphoric acid, and about 1.5-3 wt-% of the ester composition according to the invention, and water.

The preservative composition according to the invention may contain various total quantities of said ester components 1) and 2). According to one embodiment, the minimum content of ester component 1) in the preservative composition is about 0.25 wt-%, preferably about 0.5 wt-%, and most preferably about 1 wt-%. Regardless of this, the maximum content of ester component 1) is about 10 wt-%, preferably about 5 wt-%, and most preferably about 2.5 wt-%. The wt-% is given as benzoic acid and the moieties are advantageous when acid component 3) is also present.

In addition to the ingredients mentioned above, the mixture can contain other compositions that improve the preservation power, including $C_4 - C_{14}$ carboxylic acids, such as free benzoic acid, isobutyric acid, or octanoic acid, or corresponding alcohols; monoglycerides of $C_8 - C_{16}$ carboxylic acids, or other bactericidal or bacteriostatic surface-active compositions; dicarboxylic acids, such as oxalic acid, malonic acid, succinic acid, adipinic acid, pimelic acid, suberic acid, fumaric acid, or maleic acid; polycarboxylic acids, such as polyacrylic acid, or copolymer acids formed between unsaturated compositions that are polymerised with polyacrylic acid and acrylic acid; hydroxycarboxylic acids, hydroxydicarboxylic acids, or hy-

droxypolycarboxylic acids, such as sorbic acid, ascorbic acid, cinnamic acid, salicylic acid, tartaric acid, or maleic acid; complexing agents, such as EDTA, DTPA; or derivatives of all the above-mentioned acids, such as salts or esters. Furthermore, depending on the application of use, the mixture may contain metallic salts affecting
5 as preservatives, including salts of magnesium, zinc, and copper, compositions that adjust animal nutrients or the salt content of the mixture or that buffer its pH, including ammonium phosphate or lemercy salt; imidiazolindylurea, phenolic compositions and sulphonic acids, such as lignosulphonates, or aldehydes, such as formaldehyde. Furthermore, components acting as antioxidants, such as butylated hydroxyanisole or hydroxytoluene, or esters of gallic acid can be added to the mixture.
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The preservative compositions according to the invention are manufactured by simply mixing ester components 1) and 2) and possible other components, such as an acid component with each other.

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Generally, the preservative mixture is in soluble form at conventional temperatures. It can also be brought into solid form by absorbing it into large-area clay mineral, silica, or other absorption body.

20 In addition to preserving green forage, fresh cuttings, or grain, the composition according to the invention can also be used to treat or preserve other organic materials. These include cuttings of beet produced as a by-product of sugar manufacturing, liquor forage, mash, distiller's grain, by-products used as forage for food industry, forage, or forage additives, as well as organic products intended for human nutri-
25 tion. Moreover, it can be used to improve the hygiene of cattle sheds and other constructions, of plants and devices, whereby it is used to control hazardous microorganisms. Organic material preserved by using the composition according to the invention can be used, not only for nutrition, but also for raw material in industrial processes. For instance, grain preserved by the composition according to the inven-
30 tion gives increased sugar content. so that its use as raw material in alcohol manu-

facture implies increased alcohol yield. At the same time, the drying of grain is avoided, which is an energy-consuming process.

When compositions are used in products intended for human or animal nutrition,
5 their components must be chosen from compositions acceptable for additives of forage or food.

The ester mixtures of the invention are not corrosive and they do not have pungent odours. The acid solutions containing them are better in these proportions than those
10 without them.

The invention is also related to a method for preserving green forage, fresh cuttings, grain, or the like. In the method, ester component 1) which is the ester of unsubstituted or substituted benzoic acid with $C_1 - C_9$ alcohol, or a mixture of such esters,
15 and another ester component 2) that acts synergistically with said ester component 1) and is the ester of unaromatic $C_1 - C_{20}$ carboxylic acid with $C_1 - C_9$ alcohol, is added to a preservative containing an acid component 3) at the farm or some other place shortly before use or in connection with the use, so that a composition of the above-mentioned type that acts synergistically is produced as the effective mixture.

20

Examples

The invention is illustrated in the following by examples with reference to the appending drawings in which:

Fig. 1 presents the amounts of acetic acid and lactic acid in forage when the preservative contains different amounts of ethyl benzoate, propyl formate, or the mixture thereof (Example 5),
25

Fig. 2 presents the postfermentation of forage samples removed from storage, as indicated by maturity (Example 6),

Fig. 3 shows how the efficacy of acetic acid is improved in prevention of salmonella
30 when the combination of ethyl benzoate and propyl formate is used.

Example 1 (growing in solution/yeast)

Laboratory tests were conducted on the effect of the esters of benzoic acid, formic acid, and propionic acid on the growth of yeast. A YPE liquor carrier was used as the carrier, containing yeast extract. 2 % of glucose was added to the carrier. The growing was effected in Klett tubes. The growth of yeast was observed by measuring the clouding of the solution caused by the increase of microbes, by means of a Klett analyser after three days of growing. The result is an absorbency reading. The better the preventive effect, the clearer the carrier and the lower the absorbency reading.

	Preservative	amount:	0 %	0.1 %	0.5 %	1.0 %
	EB, ethyl benzoate		280	40	20	20
	PB, propyl benzoate		260	90	25	25
15	EF, ethyl formate		280	280	250	240
	PF, propyl formate		220	220	160	110
	EP, ethyl propionate		280	280	180	180
	PP, propyl propionate		260	260	130	140
	EB + PB 1:1		270	100	25	25
20	EB + EF 1:1		270	190	15	15
	EB + PF 1:1		270	40	15	15
	EB + EP 1:1		270	90	15	15
	EB + PP 1:1		270	45	15	15
	PB + PF 1:1		270	15	15	15
25	PB + EP 1:1		270	10	10	10
	PB + PP 1:1		270	10	10	10

The ethyl esters and propyl esters of the benzoic acid worked best of the individual preservatives tested. being clearly efficacious at a dose of 0.1 %. The combination of the two did not increase the efficacy. The ethyl esters and propyl esters of the

formic acid and the propionic acid alone did not yield sufficient inhibition of yeast even at a dose of 1.0 % in the test conditions. Surprising synergy was indicated when mixing an ester, which alone was not very efficacious, with benzoates, the composition obtained worked even better than the benzoate alone, even though the content of benzoate in these combinations was only half as large.

Example 2 (growing in solution/coli)

Laboratory tests were conducted on the effect of the esters of benzoic acid, formic acid, and propionic acid on the growth of Coliform bacteria. A Luria-Bertani carrier was used in the testing, its pH being adjusted to 7.5. 1 % of Coli inoculation was added, containing *E. coli*. The other procedures were similar to those in Example 1 (growing in solution/yeast). The following results were obtained:

	Preservative	amount:	0 %	0.1 %	0.5 %	1.0 %
15	EB, ethyl benzoate		105	55	25	30
	PB, propyl benzoate		90	60	80	80
	EF, ethyl formate		105	105	105	100
	PF, propyl formate		105	105	90	80
	EP, ethyl propionate		105	105	85	80
20	PP, propyl propionate		90	90	70	65
	EB + PB 1:1		100	60	55	70
	EB + EF 1:1		100	60	15	15
	EB + PF 1:1		105	65	10	10
	EB + EP 1:1		100	65	15	15
25	EB + PP 1:1		100	25	10	10

Ethyl benzoate was the most efficacious of the individual preservatives. When esters of formic acid and propionic acid, which were inefficient or low-efficient per se, were mixed with it, the result was improved as compared with ethyl benzoate alone. The best result was obtained by the mixture of ethyl benzoate and propyl

formate.

Example 3 (growing in solution/propionic acid and lactic acid/yeast)

Tests according to Example 1 were conducted by using, as preservatives, 80 wt-%
 5 of propionic acid or 80 wt-% of lactic acid which contained the total of 5 wt-% of
 ethyl benzoate (EB) and/or propyl formate (PF). The dose of the preservative in the
 solution was 0.5 wt-%. The following results were obtained:

	<u>Acid</u>	<u>Ester</u>		<u>Yeasts</u>	<u>(Klett reading)</u>	
10			Time:	1 day	2 days	3 days
	Propionic acid					
	3A	no esters		30	-	140
	3B	EB		30	-	60
15	3C	EB+PF 1:1		10	-	15
	3D	PF		10	-	95
	Lactic acid					
	3E	no esters		85	230	300 *)
20	3F	EB		50	70	90 **)
	3G	EB+PF 1:1		55	55	55 *)
	3H	PF		90	210	270 *)

Note!

25 *) Reading after adding lactic acid (0 days) = 55

***) Reading after adding lactic acid (0 days) = 40

After 3 days, there is still hardly any visible growth of yeast in Sample 3C of the
 invention which contains propionic acid and synergistic ester composition, but in
 30 Samples 3B and 3D, instead, which contain only one of the esters, and particularly
 in Sample 3A which contains no esters, the growth is substantial.

Sample 3F containing ethyl benzoate yielded a reasonable result at a dose of 0.5 wt-
 %, even though the growth had been clearly started on the second day. In sample
 35 3G according to the invention, containing lactic acid and synergistic ester composi-
 tion. in which the amount of ethyl benzoate is only half of that in Sample 3F, yeast

has not grown during 3 days enough to change the turbulence reading of the initial state. On the second day, the growth in Sample 3E with no esters and in Sample 3H containing propyl formate is very remarkable.

5 Example 4 (growing in solution/acetic acid/coliforms)

A test according to Example 2 was conducted by using acetic acid of 80 wt-% as the preservative solution, containing esters ethyl benzoate (EB) and/or propyl formate (PF). With doses of preservative of 0.5 and 0.2 wt-%, no growth could be perceived in any of the samples. With a dose of 0.1 wt-%, the following results were obtained, indicating that the growth of *E. Coli* was the slowest to start in solution 4C containing the synergistic ester composition:

15	<u>Acid</u>	<u>Ester</u>	Time:	<u>Yeasts</u> (Klett reading)		
				1 day	2 days	3 days
	Acetic acid					
	4A	no esters		65	80	80
	4B	EB		65	75	75
	4C	EB+PF 1:1		30	65	65
	4D	PF		65	80	80

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Example 5 (bag test)

Some timothy grass and meadow fescue was cut and measured into batches of 1 kg each and treated with different preservatives based on formic acid. The samples were enclosed into air-impermeable plastic bags. 5 parallel bag tests were conducted on each preservative. The doses of preservatives comprised 5 litres / a ton of forage.

The following preservative compositions were tested (the amounts are given in wt-%):

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	Ingredient	Mixture:	5/1	5/2	5/3	5/4
	Formic acid		80	78	76	74
	Phosphoric acid		2	2	2	2
	Water		18	18	18	18
5	Esters		0	2	4	6

The forages were analysed after 48 days of storage. The dry content of the samples ranged between 15.8 - 17 wt-% and the pH between 3.6 - 3.9. Acetic acid is generated in the detrimental fermentation in the forage, while fermentation resulting in lactic acid is desirable. The contents of these acids are disclosed in the appended table and in Figs. 1A and 1B:

	No.	Formula	Ester	Lactic acid	Acetic acid
				g/l	g/l
15	5A	5/1	--	65.4	8.8
	5B	5/2	EB	55.6	7.0
	5C	5/2	EB+PF	81.4	5.0
	5D	5/2	PF	71.8	11.2
20	5E	5/3	EB	62.8	7.4
	5F	5/3	EB+PF	73.8	5.0
	5D	5/3	PF	72.4	7.2
25	5H	5/4	EB	64.2	10.0
	5I	5/4	EB+PF	56.0	5.6
	5J	5/4	PF	81.2	12.2

The tables and the figures indicate that the preservatives containing the ester composition of ethyl benzoate + propyl formate according to the invention gave clearly better results than those containing only one of them. At low doses, the ester composition mainly controls detrimental fermentation; it is only at higher doses that the lactic acid fermentation is retarded.

Example 6 (postfermentation)

The forage samples according to the previous example were opened and their temperatures and the room temperature were observed for 10 days after that. The area between the temperature curves, the so-called maturity, indicates the intensity of postfermentation. The lower the maturity, the lesser the postfermentation in the sample. The results obtained as the mean value of the 5 samples are presented in Fig. 2. It is obvious that the preservative containing composition of ethyl benzoate and propyl formate clearly decreased postfermentation as compared with preservatives containing only one ester.

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Example 7 (grain preservation test)

Barley seeds were preserved by means of different preservative solutions containing acid. The moisture level of barley was 43 wt-%. The preservative was measured by spraying on an Archimedean conveyor. Two grades of dose were used, 1.8 l and 1.44 l per hundred kilos of seeds.

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The former corresponds to the recommended dose of propionic acid for seeds with such moisture content, the latter is 20 % less. The seeds were stored in barrels.

20 The following preservatives were used in the test:

1: 100 wt-% of propionic acid

2: 61 wt-% of formic acid + 30 wt-% of propionic acid + 9 wt-% of benzoic acid

3: 70 wt-% of formic acid + 20 wt-% of propionic acid + 5 wt-% of ethyl benzoate + 5 wt-% of propyl formate.

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The condition of the barley was checked after 11 weeks. The following observations were made, the numbers indicating the occurrence of mould and implying the width, length, and depth of the moulded spots in centimetres:

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Preservative	1.8 l/100 kg the amount of mould	1.44 l/100 kg the amount of mould
1 (comparison 1)	no mould	5x10x10
2 (comparison 2)	5x10x10	8x20x15
5 3 (according to the invention)	no mould	no mould.

It can be observed that the propionic acid yielded good results with the recommended dose but the incomplete dose was not sufficient. In preservative 2, the major part of propionic acid has been replaced by cheaper formic acid and benzoic acid has been used in order to compensate for the loss of preserving power. The result was unsatisfactory. In preservative 4 according to the invention, even a greater amount of propionic acid has been replaced with formic acid and the amount of benzoic acid (counted as acid) has been lowered to less than half. Nevertheless, this combination containing ethyl benzoate and propyl formate has increased the preservative power even more than what was achieved with propionic acid alone.

Example 9: Salmonella

The *Salmonella infantis* that was used in the test had originally been isolated from a chicken and cultivated in buffered peptone water. Sets of dilution were made in peptone water for the test by using different amounts of preservatives. The growth of bacteria was observed by a Bioscreen device (LabSystems, Finland). The device measures automatically the cloudiness that implies the growth of bacteria from the set of samples as a function of time. The results are shown in Figs. 3A (acetic acid) and 3B (acetic acid containing 2.5 wt-% of EB and 2.5 wt-% of PF). Inhibition of growth of Salmonella can be perceived in the acetic acid concentration of 0.5 wt-% but when 5 wt-% of the ester composition of the invention was mixed with the acetic acid, in a ratio of 1:1 of ethyl benzoate + propyl formate, the growth could be inhibited even by an amount of 0.3 wt-% of preservative.

30 Example 10 (solid preservative)

A solution was made, containing 78 wt-% of formic acid, 2 wt-% of phosphoric

acid, 1.25 wt-% of ethyl benzoate, 1.25 wt-% of propyl formate, and 17.5 wt-% of water. 55 weight fractions of molar earth (Damolin K 0.5-1 mm) was saturated with 45 weight fractions of the solution. A solid preservative was obtained which was easy to measure.

CLAIMS

1. A composition for preserving green forage, fresh cuttings, grain, or other organic material and for controlling hazardous micro-organisms, containing an ester of unsubstituted or substituted benzoic acid, **characterised** in comprising at least ester component 1) which is an ester of the unsubstituted or substituted benzoic acid with a C₁ - C₉ alcohol, or a mixture of such esters, and another ester component 2) which acts synergistically with said ester component 1) and which is an ester of an unaromatic C₁ - C₂₀ carboxylic acid with a C₁ - C₉ alcohol.
2. A composition according to Claim 1, **characterised** in that the unsubstituted or substituted benzoic acid of ester component 1) is an unsubstituted benzoic acid and/or parahydroxybenzoic acid, preferably an unsubstituted benzoic acid.
3. A composition according to Claim 1 or 2, **characterised** in that the C₁ - C₉ alcohol of ester component 1) is a C₁ - C₄ alcohol, preferably ethanol or propanol.
4. A composition according to Claim 1, 2, or 3, **characterised** in that ester component 1) is ethyl benzoate, propyl benzoate, isopropyl benzoate, or a mixture thereof.
5. A composition according to any of the preceding Claims, **characterised** in that the unaromatic C₁ - C₂₀ carboxylic acid of ester component 2) is a C₁ - C₁₀ carboxylic acid, preferably a C₁ - C₈ carboxyl acid, more preferably formic acid, propionic acid or lactic acid.
6. A composition according to any of the preceding Claims, **characterised** in that the C₁ - C₉ alcohol of ester component 2) is a C₁ - C₄ alcohol, preferably ethanol, propanol or butanol.

7. A composition according to any of the preceding Claims, characterised in that ester component 2) is propyl formate, ethyl propionate, propyl propionate, or a mixture thereof.
- 5 8. A composition according to any of the preceding Claims, characterised in that the relative weight moieties of ester component 1) and ester component 2) in the preservative composition range between about 10:1 - 1:5, preferably about 4:1 - 1:2, most preferably about 2:1 - 1:1.5.
- 10 9. A composition according to any of the preceding Claims, characterised in that it further comprises acid component 3) which is an organic or inorganic acid or a mixture thereof, possibly at least partly neutralised, or a composition that forms them, or a combination thereof.
- 15 10. A composition according to Claim 9, characterised in that acid component 3) contains an organic $C_1 - C_{20}$ acid, preferably a $C_1 - C_{10}$ acid, more preferably a $C_1 - C_4$ acid, such as formic acid, acetic acid, propionic acid, lactic acid, or a mixture thereof, preferably formic acid.
- 20 11. A composition according to Claim 10, characterised in that acid component 3) is a water solution of 10-100, preferably 30-100, more preferably 60-100 wt-%.
12. A composition according to Claim 9, 10 or 11, characterised in that acid component 3) contains phosphoric acid or a derivative or mixture thereof.
- 25 13. A composition according to any of the preceding Claims, characterised in that the minimum content of ester component 1) in the preservative composition, counted as benzoic acid, is about 0.25 wt-%, preferably about 0.5 wt-%, most preferably about 1 wt-%.

14. A composition according to any of the preceding Claims, characterised in that the maximum content of ester component 1) in the preservative composition, counted as benzoic acid, is about 10 wt-%, preferably about 4 wt-%, most preferably about 2.5 wt-%.

15. A composition according to any of Claims 9-14, characterised in containing about 1.5 - 3.0 wt-% of a mixture of ester component 1) and ester component 2), about 70-90 wt-% of formic acid, and about 0-10 wt-% of orthophosphoric acid as acid component 3), and water.

16. A composition according to any of the preceding Claims, characterised in being in the form of a solution.

17. A composition according to any of the preceding Claims, characterised in that it is made into a solid form, preferably by absorbing into a carrier, such as a large-area clay mineral.

18. A method for preserving green forage, fresh cuttings, grain or other vegetable material, characterised in that ester component 1) which is an ester of unsubstituted or substituted benzoic acid with a $C_1 - C_9$ alcohol, or a mixture of such esters, and another ester component 2) that acts synergistically with said ester component 1) and which is an ester of an unaromatic $C_1 - C_{20}$ carboxylic acid with a $C_1 - C_9$ alcohol, is added to a suitable gaseous, aqueous or solid medium, preferably to a liquid preservative composition which may contain acid component 3), at a farm or other place of use shortly before use or in connection with the use, so that a synergistically-acting composition of the above-mentioned type is generated as the effective mixture.

19. The use of the composition according to any of Claims 1-17 or of that manufactured by means of the method according to Claim 18 as an animal forage additive.
20. The use of the composition of any of Claims 1-17 or of that manufactured by
5 means of the method according Claim 18 to improve the hygiene of cattle sheds or the like and/or to disinfect devices and equipment.

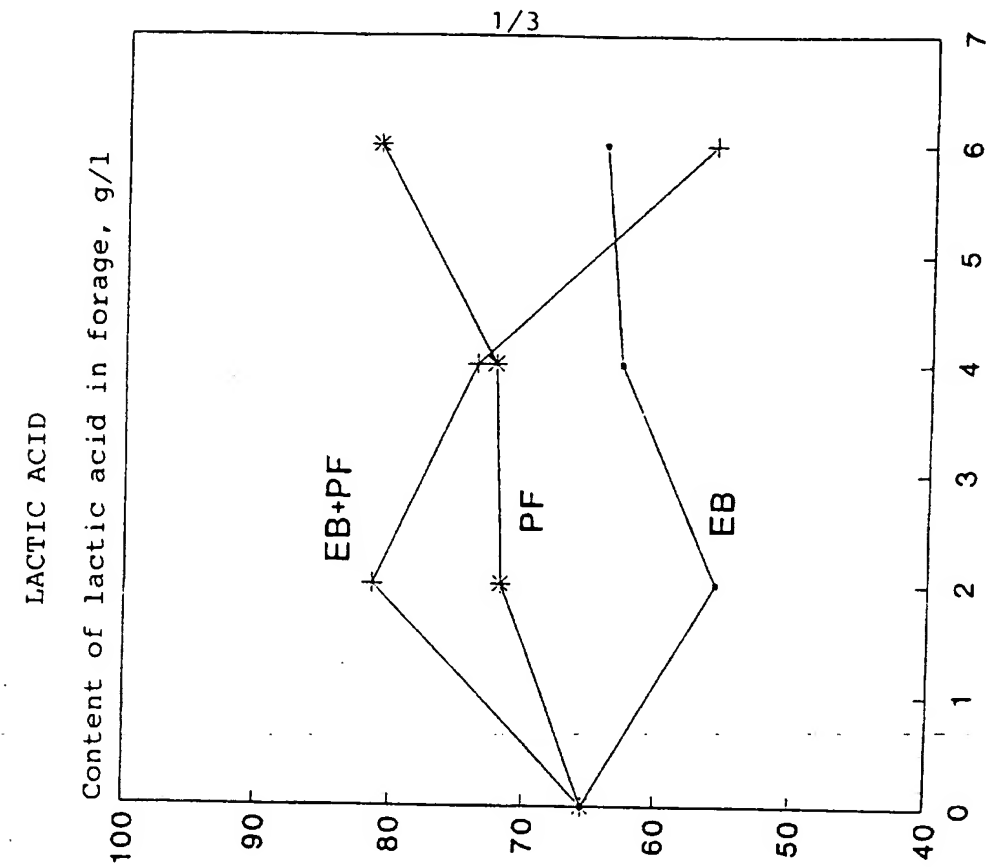


FIG. 1B Content of ester in preservative, %

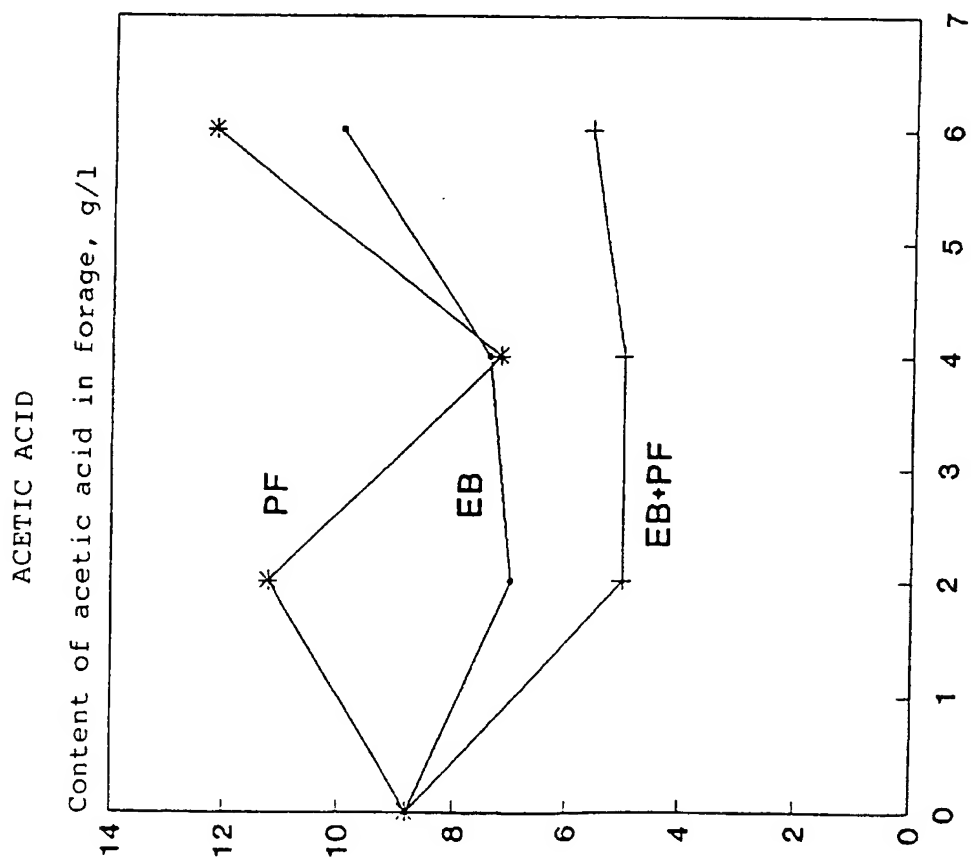


FIG. 1A Content of ester in preservative, %

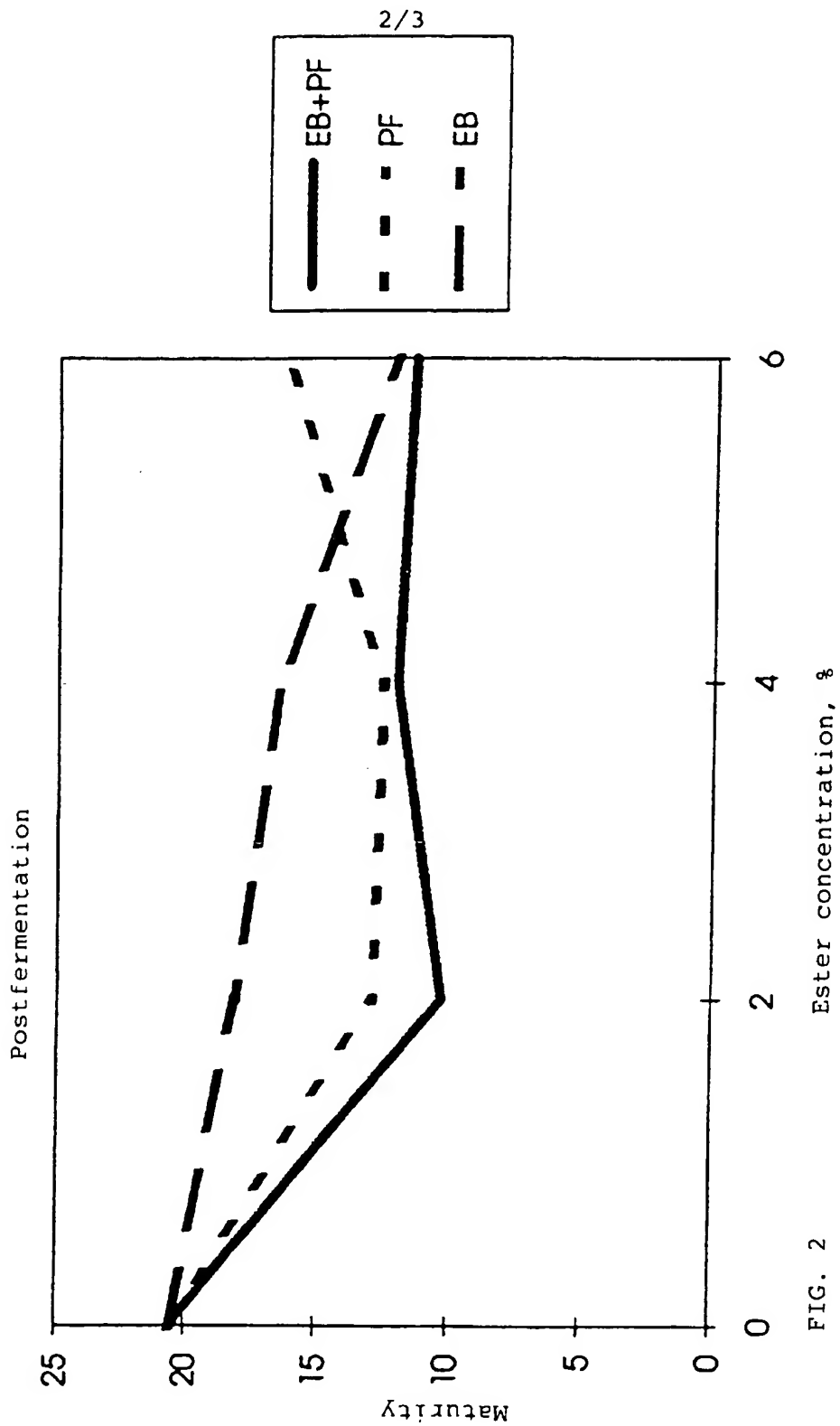


FIG. 2

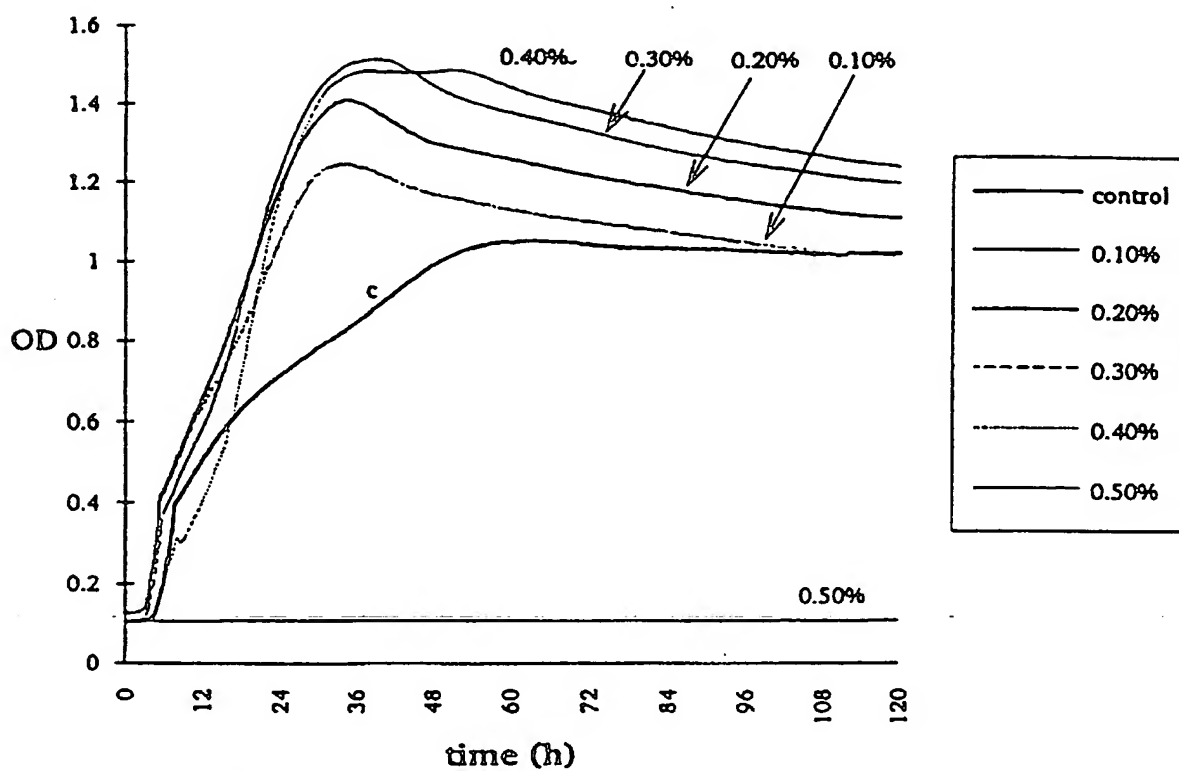
3/3
AS 5

FIG. 3A Acetic acid

AS 11

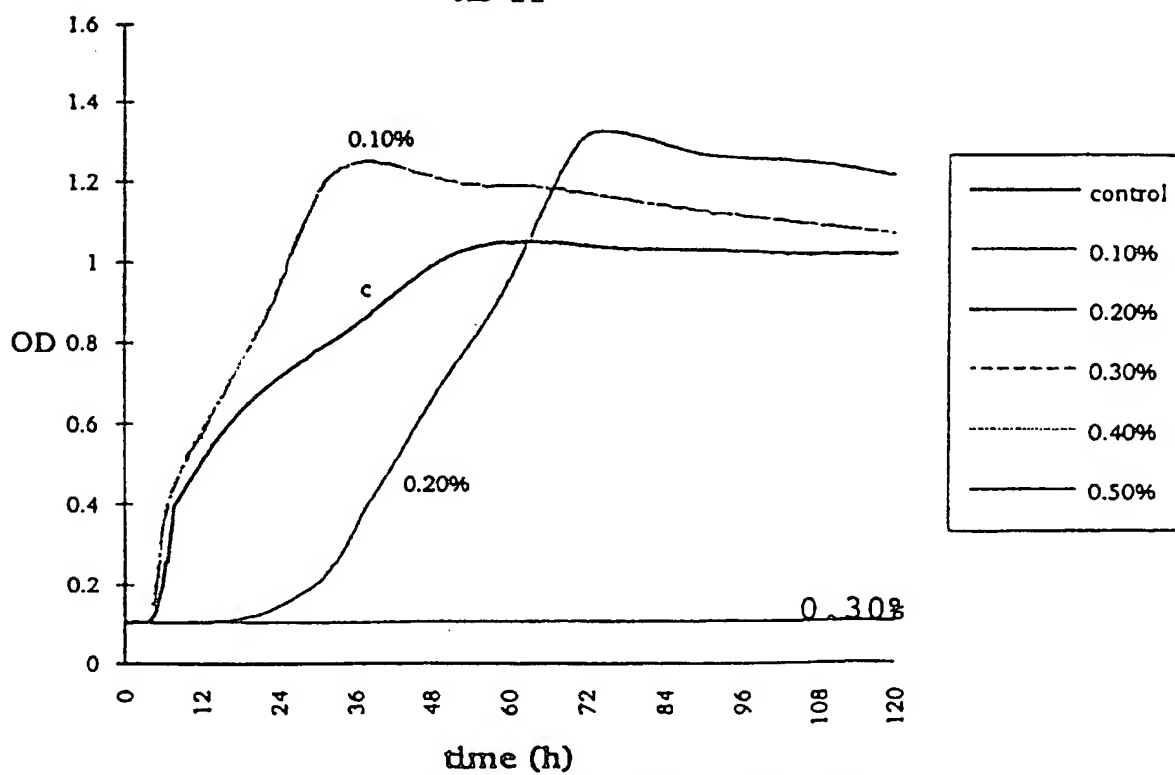


FIG. 3B Acetic acid + 2,5% EB + 2,5% PF

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 96/00065

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A01N 37/02, A01N 37/06, A01N 37/10, A23K 3/03, A23L 3/3517
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A01N, A23K, A23L, A23B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9316611 A1 (KEMIRA OY), 2 Sept 1993 (02.09.93) --	1-20
X	WO 9221239 A1 (ECOLAB INC.), 10 December 1992 (10.12.92) --	1-20
X	WO 9316603 A1 (KEMIRA OY), 2 Sept 1993 (02.09.93) -- -----	1-20

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INTERNATIONAL SEARCH REPORT

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WO-A1- 9316611	02/09/93	AU-A- 3502193 EP-A- 0626812 FI-B,C- 95195 LT-A- 706 LT-B- 3474 LV-B- 10563 NO-A,D- 943168	13/09/93 07/12/94 29/09/95 31/01/95 25/10/95 20/02/96 26/08/94
WO-A1- 9221239	10/12/92	AU-B- 665548 AU-A- 2001192 EP-A- 0588912 JP-T- 6508612 NZ-A- 240355 US-A- 5336500 US-A- 5419908	11/01/96 08/01/93 30/03/94 29/09/94 27/09/94 09/08/94 30/05/95
WO-A1- 9316603	02/09/93	AU-A- 3502293 LT-A- 362	13/09/93 15/07/94

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